bacterioplankton (Fuhrman, J.A., et al. 1993), soil (Nakatsu, C. et al. 2000) as well as hydrothermal environment (Moyer, C.L., et al. 1994).

Denaturing gradient gel electrophoresis (DGGE) technique is now routinely used in many microbiological laboratories worldwide as molecular tool to compare the diversity of microbial communities and to monitor population dynamics. Separation fragments in DGGE is based on the decreased electrophoretic mobility of partially melted double-stranded DNA molecules in polyacrylamide gels containing a linear gradient of DNA denaturants (a mixture of urea and formamide). Molecules with different sequences may have a different melting behavior, will stop migrating at different positions in gel. More information about the identity of community members can be obtained by hybridization analysis of DGGE patterns with polynucleotide probes or direct sequencing of reamplified DGGE bands (Muyzer, G., 1999).

Our objective in this study was to describe the diversity and constructing the phylogeny tree of bacterial community inhabiting one of hot spring at Gedongsongo field of Ungaran volcano, central Java. The extensive study has been done to this field according their geophysical and geochemistry study (Widarto et al, 2003). The hydrothermal system of Gedongsongo field consists of some hot-springs, hot-pools, and steaming ground. However, there has no report according their microbial diversity. Aminin et al (2003) has been isolate and characterize the extracellular proteolitic enzyme from thermophilic bacteria of Gedongsongo hot spring, but has not been characterize the species of this thermophile.

2. Experimental

Description of the WGS2 hot spring and sampling procedure. WGS2 hot spring belong to Gedongsongo field that located in the southern flank of Ungaran volcano, central Java (110°20'23,4"E; 07°12'08,5"S; and the altitude 1400m). This hot spring has temperature 70,2°C and pH 5,85. The microbial sample was collected on 11 June 2004 from one point (the center) of WGS2 hot spring. The microbial diversity studies were approached using two methods: cultivation and filtration. The cultivation procedure was carried out using two kinds of minimal mediums which are MM_1 and MM_2. The formula of MM_1 medium is like ½ LB and MM_2 like NB medium, except the water which were used is the spring water. These medium were cultured at 60°C without shaking for 24 hours. For the filtration procedure, the water sample was kept in a sterile plastic container (2L) and brought to the laboratory immediately within 2 hours. Afterwards, cells were harvested by filtration of 1 L volumes of spring water gently through 0,2 µm (nominal pore size) Millipore filters. Resulting filtrates containing bacterial communities were stored at -20°C until DNA was extracted.

Nucleic acid extraction. Each microbial sample which is from cultivation or filtration was pellet by centrifugation. DNA was extracted using slight modifications of a method described by Klijn et al. (1991). The pellet was suspended in 200 µl of 10 mM Tris HCl buffer (pH 8.0) containing 8mg/ml of lysozyme and incubation at 37°C for 1 h, the cells were lysed by adding 200 µl lysis buffer containing 2% sodium dodecyl sulfate, 0,8 mg/ml proteinase K and 200 mM EDTA pH 8,0. The lysis process was done by incubation at 50°C for 30 min. The purification step was carried out by adding 200 µl of chloroform:isoamil-alcohol (24:1), vortex and centrifugation at 1300 g, 30 second. The upper solution was moved to clean tube. This step was done 3 times. Subsequently, the DNA was precipitated by adding 60 µl of 3 M sodium acetate and 1 ml of 96% ethanol (stored at -20°C). After centrifugation, the DNA pellet was dissolved in 10 mM Tris HCl buffer (pH 8.0). The DNA pellet was washed with 70% ethanol and finally dissolved in 50 µl of TE buffer (10 mM Tris HCl [pH 8.0], 1 mM EDTA).

PCR. The amplification of partial 16S rRNA genes of the domain *Bacteria* were as previously described (Ferris, et al, 1996). One primer (P1) complements a region conserved among members of the domain *Bacteria* (*Escherichia coli* positions 1055 to 1070). The other primer (P2) is based on a universally conserved region (*E. coli* positions 1392 to 1406; and incorporates a 40-base GC clamp). The primer sequence:

P1: 5'-ATGGCTGTCGTCAGCT-3'